SUPPLEMENTAL INFORMATION

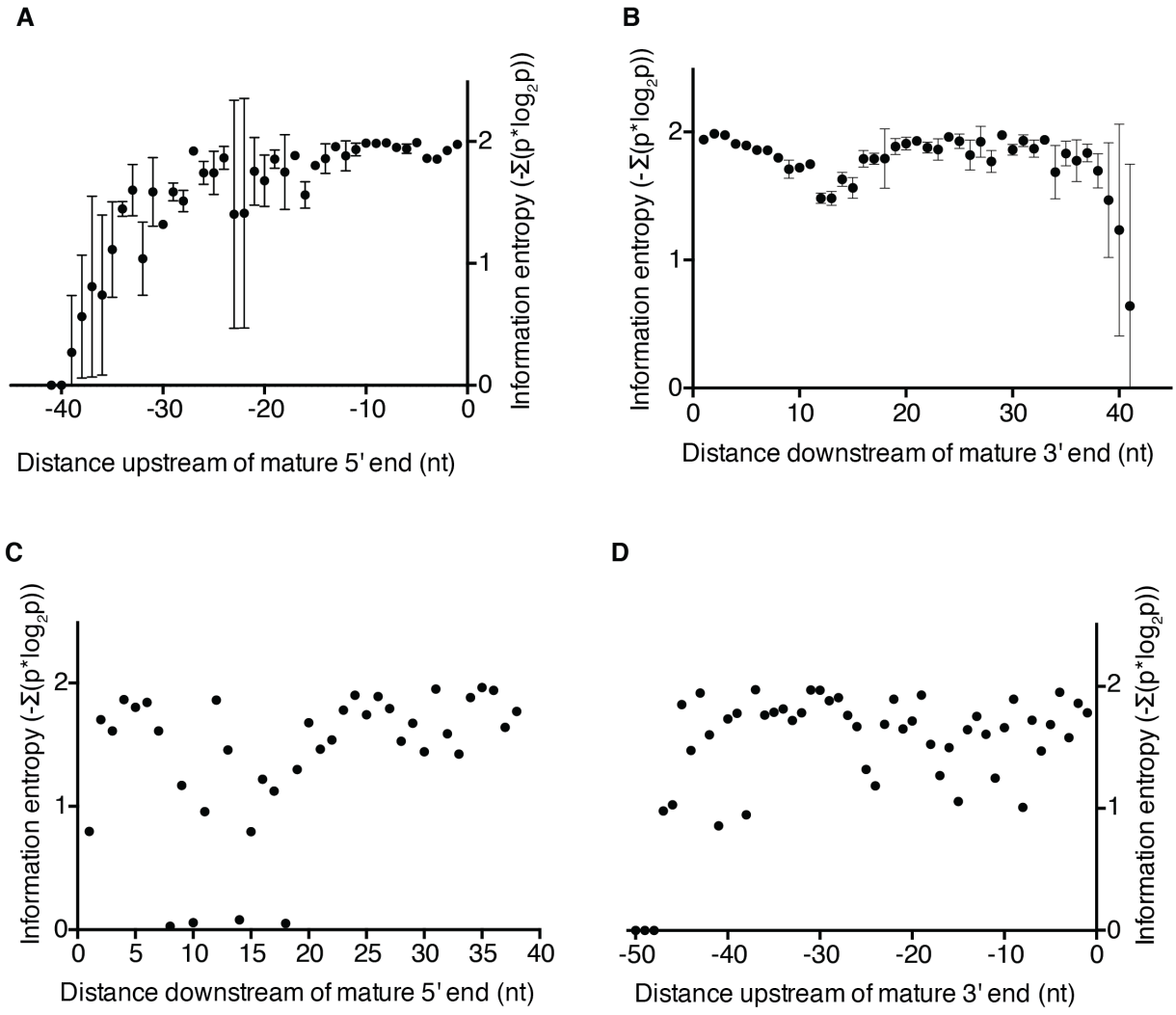

Figure S1. Information entropy in pre-tRNA segments and mature body. Related to Figure 1.
(A,B) Information entropy ($-\Sigma(p*\log_2 p)$, where p is the frequency of each nucleotide at a given position) was calculated using read evidence from hydro-tRNAseq (four replicates) for the 5' leader and 3' trailers of all pre-tRNAs with positions centered at the 5' and 3' ends of mature tRNAs. (C,D) Same as before, but using the reference sequence of mature tRNAs.

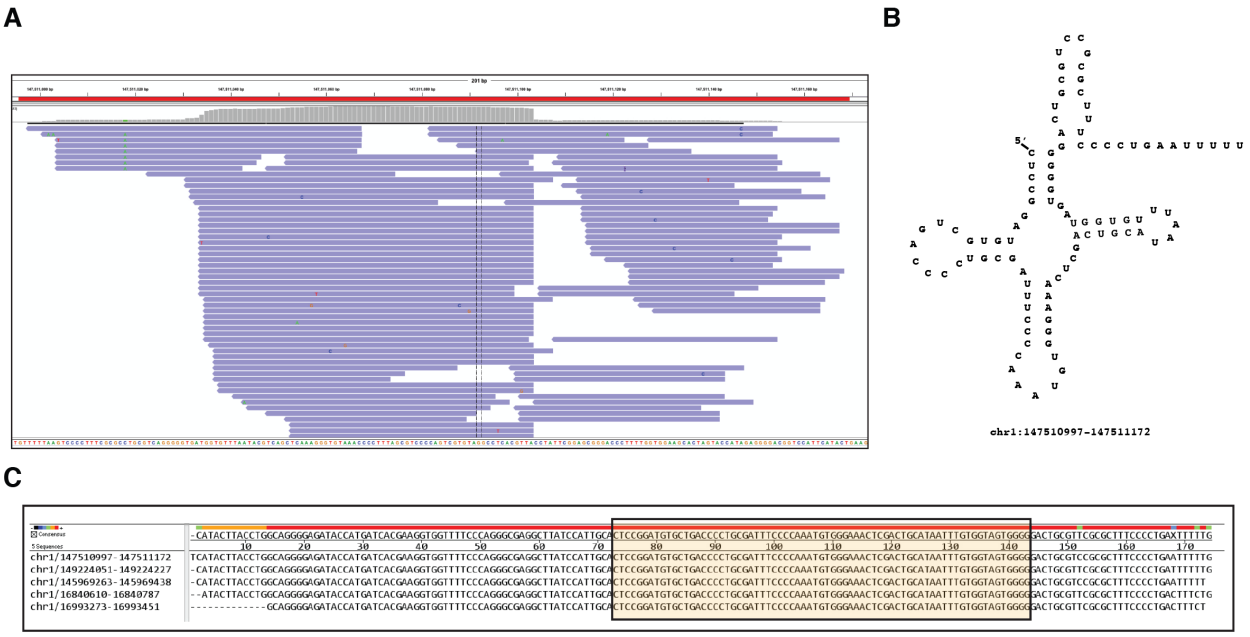

Figure S2. U1 snRNA genes detected by hydro-tRNAseq. Related to Figure 1.
(A) IGV snapshot of a genomic region with length less than 100 nt and coverage by at least 50 reads at every position that adopts a cloverleaf structure as shown in (B) and corresponds to an annotated U1 snRNA. (C) Multiple genomic locations that give rise to the same U1 snRNA sequence shown in (A); the U1 snRNA sequence is highlighted.

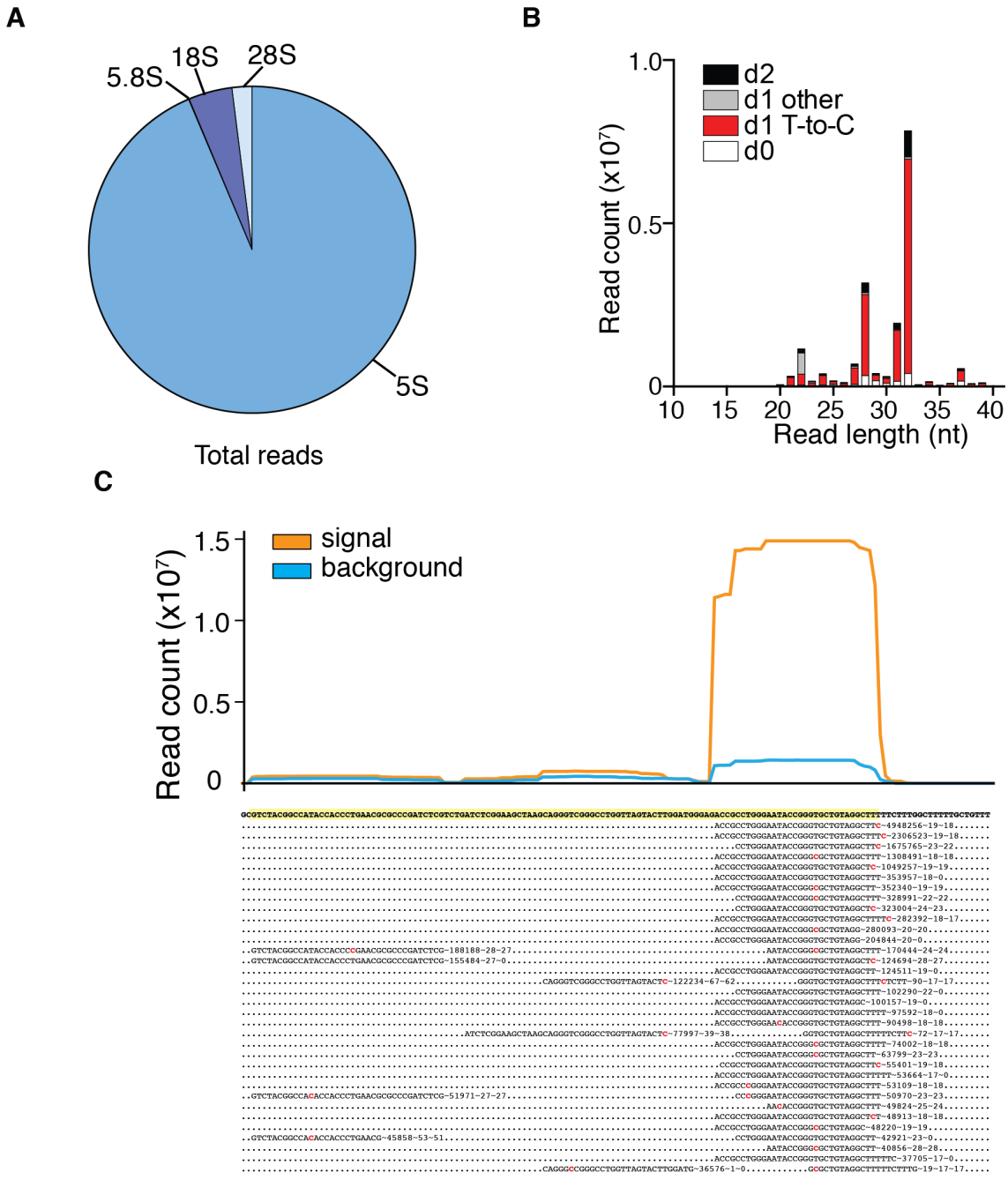

Figure S3. SSB binds 5S rRNA. Related to Figure 2.
(A) Assignment of reads from SSB PAR-CLIP to rRNAs. (B) Abundance of reads mapped to 5S rRNA with 0, 1 or 2 mismatches (d0, d1, d2) as a function of read length; reads with T-to-C mismatches are separated (red) from the rest of the reads with one mismatch (gray). Read length and number of reads are represented on the x- and y-axis, respectively. (C) Read alignments corresponding to 5S rRNA. Crosslinked positions are shown in red. The read count is shown next to each read sequence, followed by the total mapping positions, and the mapping positions that contain a T-to-C transition. Crosslinked and non-crosslinked read coverage is graphically represented.

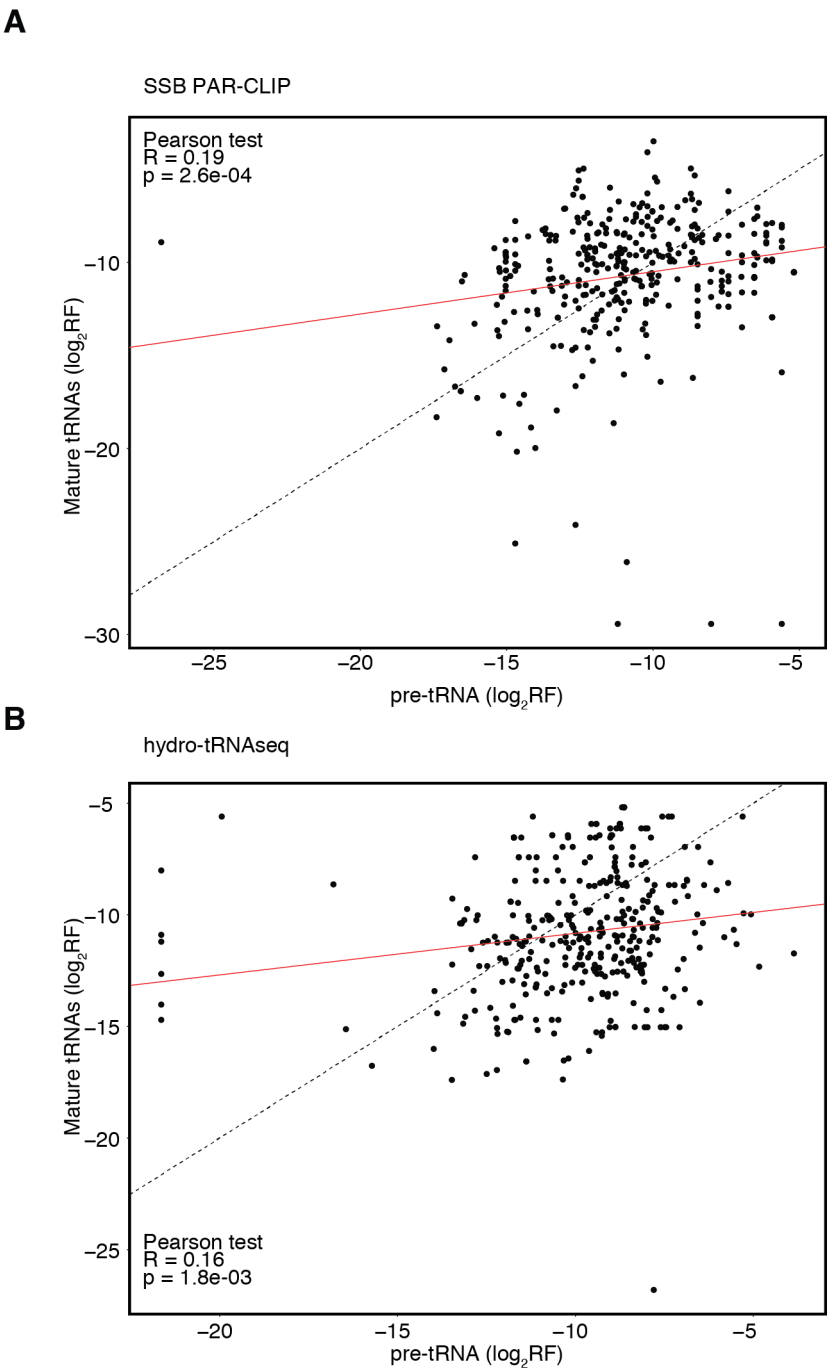

Figure S4. Correlation between pre-tRNA and mature tRNA read frequencies. Related to Figure 3.
Correlation of relative read frequencies (log_2-transformed) between pre-tRNA (x-axis) and mature tRNAs (y-axis) for SSB PAR-CLIP (A) and hydro-tRNAseq (B). Correlation was calculated using the Pearson test. Linear fit is shown in red. The y=x line (dotted) is shown for comparison.

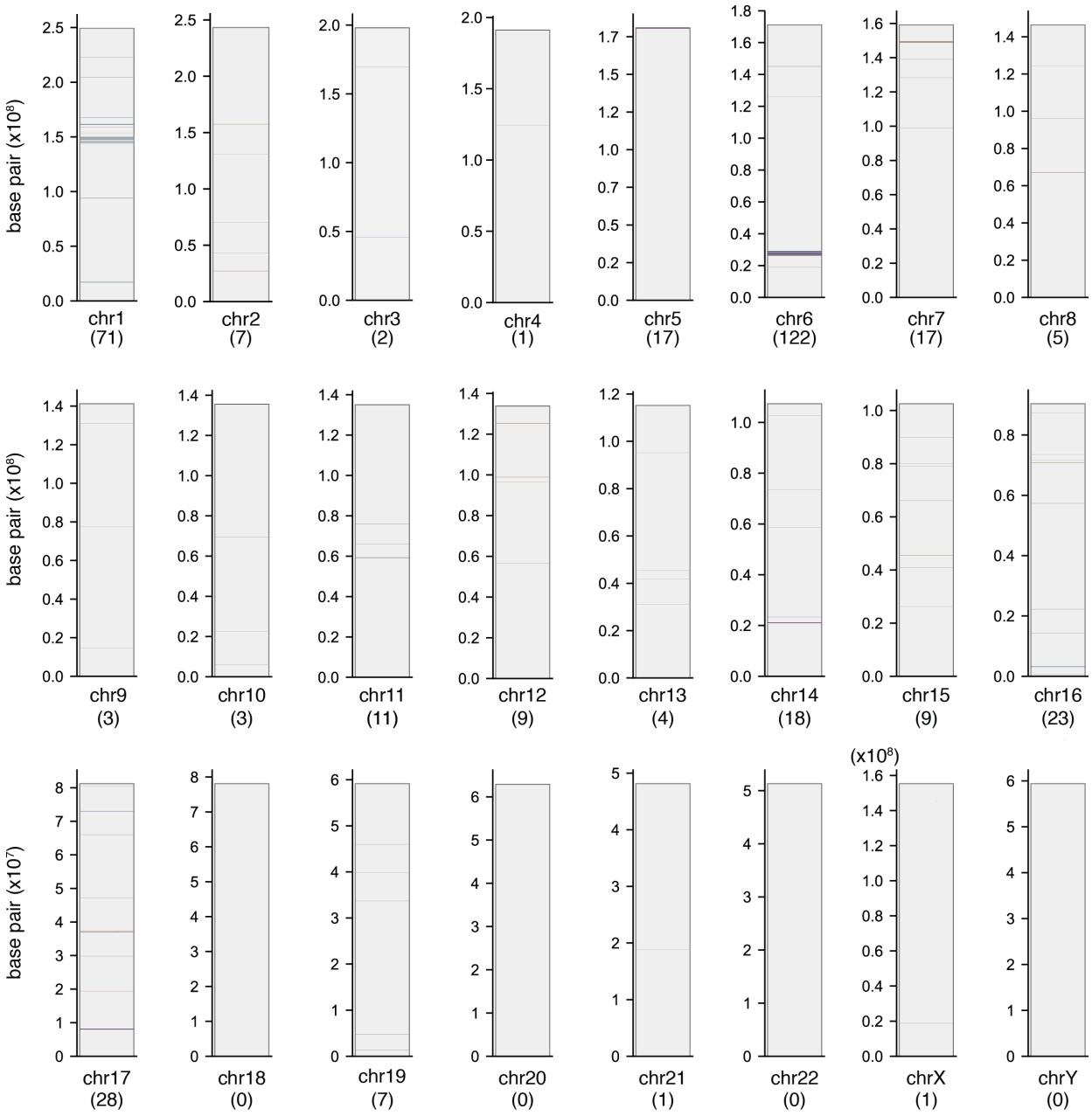

Figure S5. Genomic distribution of tRNA genes. Related to Figures 3 and 4.
The location of each expressed tRNA gene is represented by horizontal lines with respect to the location within each chromosome. Each isotype is represented by a different color. Each chromosome is scaled, to facilitate visualization with its beginning shown as base pair 0. The number of expressed tRNA genes for each chromosome is shown in parenthesis below the chromosome number. Union of all detected tRNA genes is shown (n = 359).

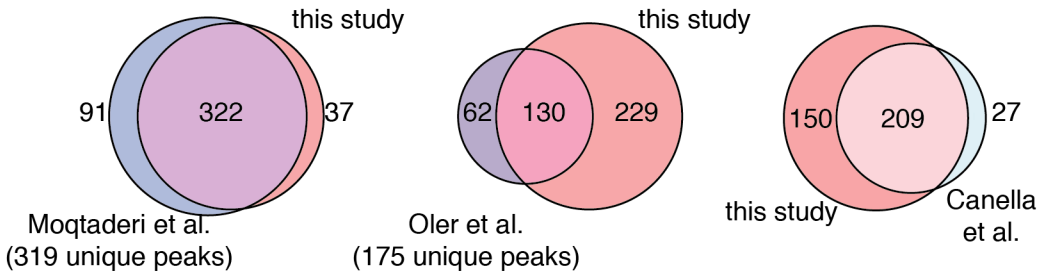

Figure S6. Overlap with POLR3 ChIP-seq studies. Related to Figure 3.
The overlap of detected genes between this study and three, previously published, POLR3 ChIP-seq is shown (Canella et al., 2010; Moqtaderi et al., 2010; Oler et al., 2010). The union of all genes detected by hydro-tRNAseq and SSB PAR-CLIP (n = 359) is included. The number of tRNA genes detected by POLR3 ChIP-seq is reflected and stated in the Venn diagrams, while the number of unique peaks that covered the given genes is indicated in parentheses below the study's reference. Due to the large size of genomic regions in the first two studies, many tRNA genes fell within the same peak and were thus indistinguishable. Coordinates of peaks were converted from hg18 to hg19, resulting in slightly different numbers compared to the original publications.

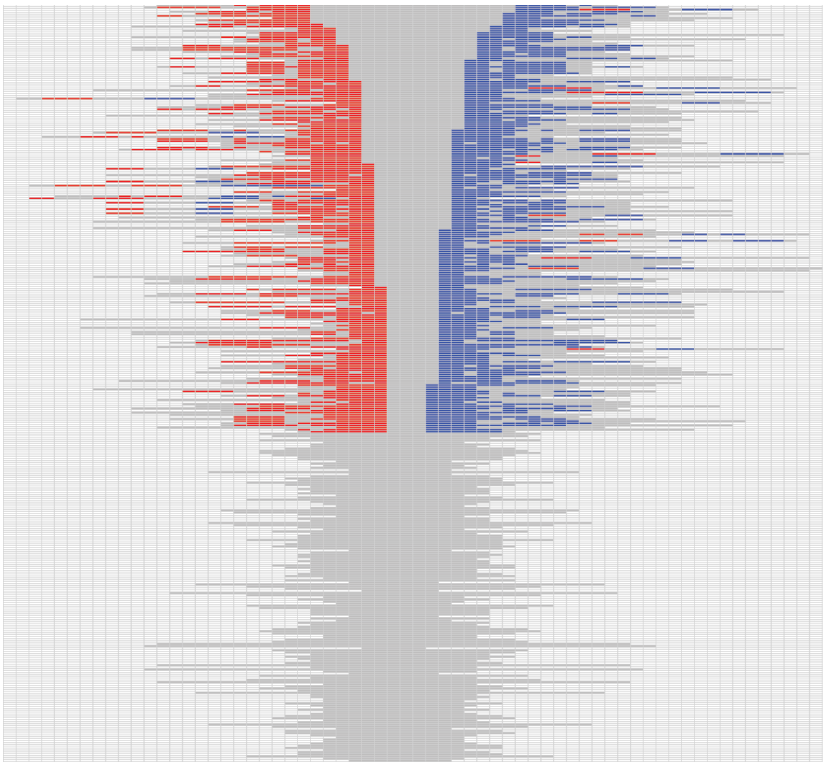

Figure S7. Predicted structures of precursor tRNA 3' trailers with read evidence in SSB PAR-CLIP. Related to Figure 6.

Every colored box indicates one nucleotide. Red and blue reflect the 5' and 3' parts of a duplex, respectively, whereas gray indicates non-base-paired nucleotides. About 1/2 of all tRNA trailers lack any predicted structure (lower part of the figure). The predicted stemloops do not have a uniform stem or loop length. Folding was performed using the ViennaRNA folding package (Lorenz et al., 2011).

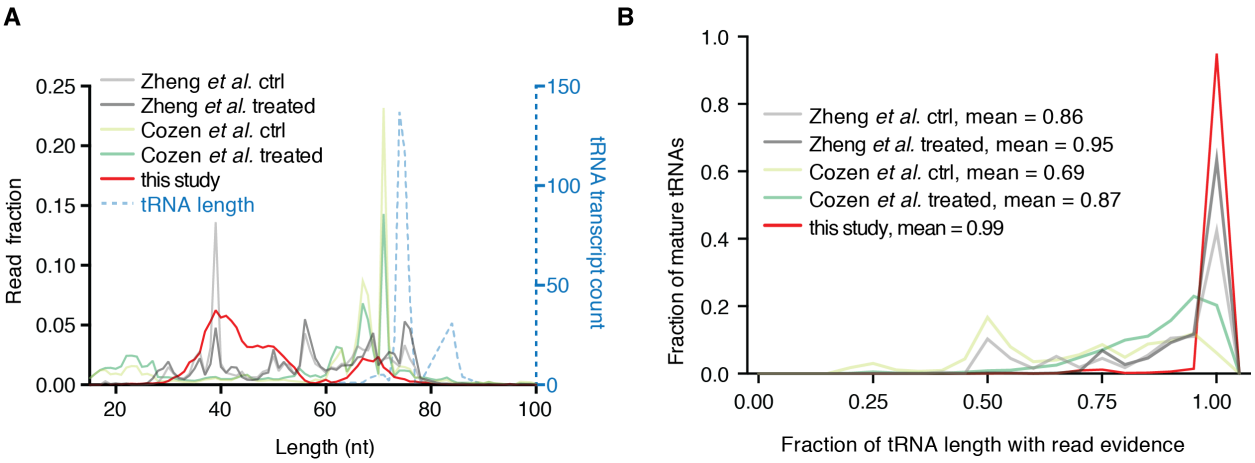

Figure S8. Read length distribution and mature tRNA read coverage for hydro-tRNAseq and dealkylating sequencing methods. Related to Figures 1 and 4.

(A) The fraction of reads with a given length is indicated for hydro-tRNAseq, as well as untreated and treated samples from dealkylating methods (Cozen et al., 2015; Zheng et al., 2015). The distribution of tRNA lengths is shown in dotted blue lines on the right y-axis for comparison. (B) Histogram representing the fraction of normalized mature tRNA transcript length with ungapped and overlapping read evidence in hydro-tRNAseq and tRNA sequencing methods employing dealkylation steps (control and subjected to treatment). The mean fraction is indicated next to each method.

Supplementary References

Lorenz, R., Bernhart, S.H., Höner Zu Siederdissen, C., Tafer, H., Flamm, C., Stadler, P.F., and Hofacker, I.L. (2011). ViennaRNA Package 2.0. Algorithms Mol Biol *6*, 26.